Krebs solution of the following composition (mM): NaCl (118), KCl (4.75), KH_2PO_4 (1.19), $CaCl_2$ (2.54), $NaHCO_3$ (25), $MgSO_4$ (1.2), and glucose (11). The mouse vas deferens[48] (MVD) was incubated at 25 °C in a solution of the following composition (mM): NaCl (118), KCl (4.75), KH_2PO_4 (0.95), $CaCl_2$ (2.54), $NaHCO_3$ (25), glucose (11), tyrosine (0.25), and ascorbic acid (0.1). The bath solution was gassed with 95% O_2–5% CO_2. The tissue was stimulated at 0.1 Hz by current applied through a pair of platinum electrodes, and the electrically stimulated isometric contractions were recorded. [Leu^5]Enkephalin, DALE, or ENAPE was added to the bath. The IC_{50} was determined from regression analysis at several doses over and below 50% inhibition of the electrically stimulated twitch. Geometric means of IC_{50} values from several experiments were obtained.

Photolysis of Guinea Pig Ileum in the Presence of ENAPE. A GPI strip was put in a Pyrex organ bath and incubated in the same bath solution (5 mL) used in the bioassay experiment. ENAPE was added in a final concentration of either 10 or 100 nM. The preparation was photolyzed at room temperature with a Panasonic electronic flash (Model PE-3000) by direct exposure to the Xenon lamp after the plastic window was removed. The flash pulse was in the millisecond range, and the time interval of each flash was about 10 s. The strip was exposed to the flash at a distance of 5 cm. One hundred flashes were sufficient to photolyze the azido group of ENAPE completely. Then the bath solution was replaced several times with fresh solution. The response of the muscle strip to 90 nM [Leu^5]-enkephalin was recorded before and after photolysis. As a control, similar bioassay experiments were done under the same conditions with 5 and 20 nM previously photolyzed ENAPE. Photolysis of the GPI in the absence of ENAPE was also done as another control.

Effect of DALE on the Photolysis of Guinea Pig Ileum with ENAPE. After the addition of DALE (final concentration 20 μM) to the GPI strip in the Pyrex bath, the incubation mixture was subjected to 10 nM ENAPE and exposed to 100 flashes. The bath solution was replaced, and the electrically stimulated contractions were recorded. Higher doses of DALE and ENAPE (final concentrations 40 μM and 100 nM, respectively) were also studied. The response to 180 nM [Leu^5]enkephalin was recorded before and after photolysis as a measure of the muscle strip response.

Photolysis of Mouse Vas Deferens. Irradiations of MVD strips were done similarly to those of the GPI, except that the bath solution for the MVD bioassay was used. Doses of ENAPE and DALE were 10 nM and 10 μM, respectively.

Acknowledgment. This work was supported in part by Grants in Aid for Scientific Research (57213002, 58105003, 58470116) from the Ministry of Education, Science and Culture, Japan.

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(Acylaryloxy)acetic Acid Diuretics. 5. [(2-Alkyl- and 2,2-Disubstituted-1,3-dioxo-5-indanyl)oxy]acetic Acids

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Investigation of the chemistry of the potent uricosuric diuretic indacrinone (MK-196) prompted the synthesis of a series of 3-oxo derivatives, i.e., the indan-1,3-diones. In general, both pharmacological parmameters (uricosuria and diuresis) were significantly less pronounced with the 1,3-diones than with the parent 1-oxo compounds.

In the first paper of this series[1] we reported on a number of [(2-alkyl- and 2,2-dialkyl-1-oxo-5-indanyl)oxy]acetic acids, which were shown to possess a high order of diuretic activity in rats, dogs, and chimpanzees and pronounced uricosuria in chimpanzees. Subsequent publications have dealt with the replacement of one of the 2-alkyl substituents with aryl moieties,[2] a series of 5-acylbenzofuran-2-carboxylic acids,[3] and a series of indeno[5,4-*b*]furans.[4] In this report, we describe the synthesis of [(1,3-dioxo-5-indanyl)oxy]acetic acids and the effects of this structural modification on saluretic and uricosuric activity.

Chemistry. Three synthetic routes to the indan-1,3-diones were employed. When 2,3-dimethylanisoles or 2-methyl-3-chloroanisoles or the corresponding oxyacetic acid esters were employed as starting materials, the Friedel–Crafts reaction with a suitably substituted malonyl chloride provided the indandiones. Ether cleavage of the anisoles, **1**, gave the phenolic derivatives, **2**, which were alkylated with bromoacetic acid ester and then hydrolyzed to the desired product, **4**. This sequence is shown in Scheme I.

Scheme I

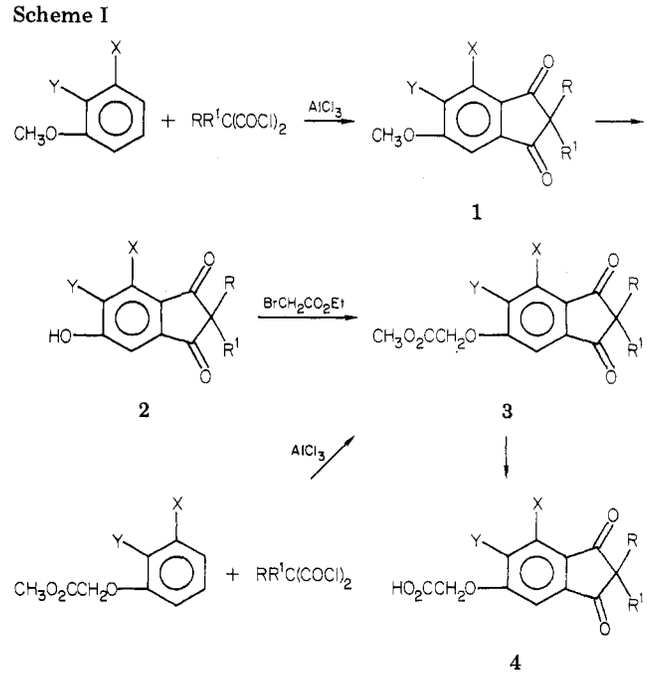

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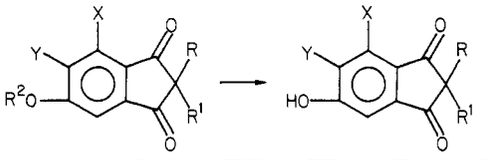
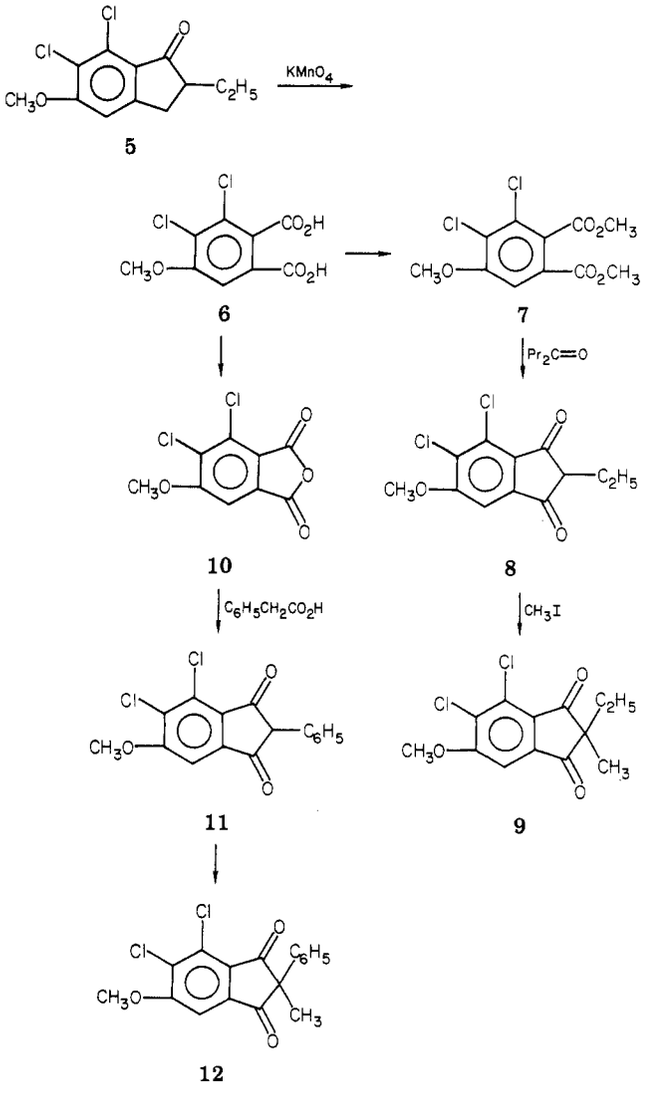
Table I. Physical Properties of Methoxy and Hydroxyindandiones



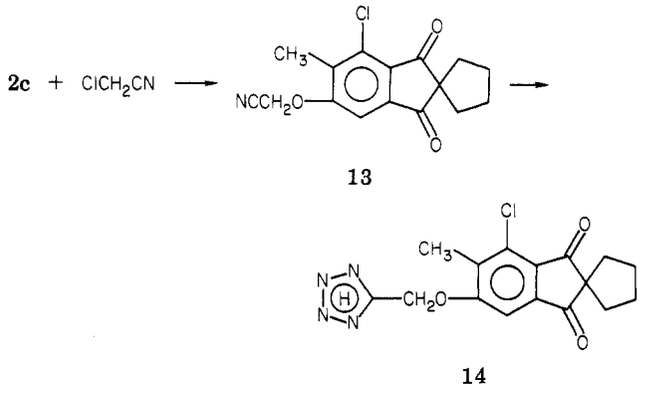
no.	X	Y	R	R^1	R^2	recrystn solvent	mp, °C	emp formula	anal.
1a	CH_3	CH_3	C_2H_5	C_2H_5	CH_3	petr ether	80–81	$C_{16}H_{20}O_3$	C, H
1b	Cl	CH_3	CH_3	c-C_5H_9	CH_3	[a]			
1c	Cl	CH_3		$-(CH_2)_4-$	CH_3	EtOH–H_2O	156–157	$C_{15}H_{15}ClO_3$	H; C[b]
2a	CH_3	CH_3	C_2H_5	C_2H_5	H	MeOH–H_2O	141–142	$C_{15}H_{18}O_3$	H; C[c]
2b	Cl	CH_3	CH_3	c-C_5H_9	H	AcOH–H_2O	221–222	$C_{16}H_{17}ClO_3$	C, H
2c	Cl	CH_3		$-(CH_2)_4-$	H	AcOH–H_2O	297–298	$C_{14}H_{13}ClO_3$	C, H
2d	Cl	Cl	CH_3	C_2H_5	H	AcOH	246	$C_{12}H_{10}Cl_2O_3$	C, H
2e	Cl	Cl	CH_3	C_6H_5	H		221–226	$C_{16}H_{10}Cl_2O_3 \cdot {}^1/_2H_2O$	C, H
8	Cl	Cl	H	C_2H_5	CH_3	MeOH–H_2O	153–154	$C_{12}H_{10}Cl_2O_3$	C, H, Cl
9	Cl	Cl	CH_3	C_2H_5	CH_3	EtOH	157	$C_{13}H_{12}Cl_2O_3$	C, H, Cl
11	Cl	Cl	H	C_6H_5	CH_3	EtOH	175–178	$C_{16}H_{10}Cl_2O_3$	H; C[d]
12	Cl	Cl	CH_3	C_6H_5	CH_3	C_6H_6–C_6H_{12}	174–175.5	$C_{17}H_{12}Cl_2O_3$	H; C[e]

[a] Not purified. [b] C: calcd, 64.64; found, 65.12. [c] C: calcd, 73.15; found, 73.65. [d] C: calcd, 59.84; found, 59.22. [e] Calcd, 60.92; found, 60.23.

Scheme II



Scheme III



In the second synthetic sequence (Scheme II) to obtain indandiones bearing two nuclear chlorine substituents, indanone **5** was oxidized to the phthalic acid **6**, esterified, and then allowed to react with 4-heptanone in the presence of sodium hydride.[5] The 2-ethylindandione, **8**, thus obtained could then be alkylated with methyl iodide, resulting in **9**. The third synthetic route consisted of converting **6** to the phthalic anhydride, **10**. Reaction with phenylacetic acid[6] gave the 2-phenylindandione, **11**, which was alkylated with methyl iodide, affording **12**. The remaining steps to **4** are shown in Scheme I. There is considerable evidence that the 5-tetrazolyl moiety serves as a carboxy surrogate in some medicinal agents;[7] therefore, **14**, the 5-tetrazolyl analogue of **4d**, was prepared by the reaction sequence shown in Scheme III.

Structure–Activity Relationships. Salidiuretic activity was determined for the compounds described in this paper in one or more of three species. This involved generating oral rat, intravenous and oral dog, and intravenous and oral chimpanzee data. For the compounds evaluated in chimpanzees, uricosuric data also were obtained.

Obviously, the novel contribution to the structure–activity relationship (SAR) knowledge of the compounds of this paper is the introduction of the 3-oxo group. The known uricosuric activity of certain nondiuretic indan-1,3-diones[8] was expected to possibly increase the relative

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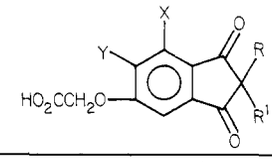
Table II. Physical Properties of [1,3-Dioxo-5-indanyl)oxy]acetic Acids

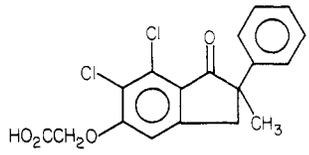


no.	X	Y	R	R^1	recrystn solvent	mp, °C	emp formula	anal.
4a	CH_3	CH_3	C_2H_5	C_2H_5	$AcOH-H_2O$	134–136	$C_{17}H_{20}O_5$	C, H
4b	CH_3	CH_3	H	C_2H_5	$AcOH-H_2O$	187–193	$C_{15}H_{16}O_5$	C, H
4c	Cl	CH_3	CH_3	c-C_5H_9	$AcOH-H_2O$	178–180	$C_{18}H_{19}ClO_5$	C, H
4d	Cl	CH_3		$-(CH_2)_4-$	$AcOH-H_2O$	204–205	$C_{16}H_{15}ClO_5$	C, H
4e	Cl	CH_3	C_2H_5	C_2H_5	$AcOH-H_2O$	167–168.5	$C_{16}H_{17}ClO_5$	C, H
4f	Cl	Cl	CH_3	C_2H_5	CH_3NO_2	214	$C_{14}H_{12}Cl_2O_5$	C, H
4g	Cl	Cl	CH_3	C_6H_5	CH_3NO_2	182–185	$C_{18}H_{12}Cl_2O_5 \cdot H_2O$	C, H

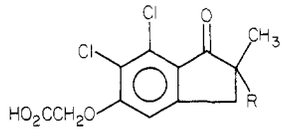
uricosuric activity of the compounds prepared for this study over their 3-deoxo counterparts.

Although the number of compounds described is small, attempts will be made to compare the members among themselves and, wherever possible, with the 3-deoxo analogues reported previously.[1-4]

Oral Rat Assays. The results of this study are recorded in Table III. In studies with the 6-methyl-7-chloro compounds (**4c–e**), the 2,2-diethyl compound **4e** was the most efficacious salidiuretic, having an activity in the range of MK-196 and furosemide and greater than hydro-



MK-196



12b, R = Et
f, R = c-C_5H_9

chlorothiazide. The 6,7-dichloro-2-methyl-2-ethyl compound **4f** was only slightly less active. In the oral rat assay, compound **4f** is the only compound where the effect of the 3-oxo group can be compared directly with its 3-deoxo compound reported previously[1] (compound **12b** in that series). It appears that **4f** is somewhat less salidiuretic than its 3-deoxo counterpart.

Dog Assays. (a) Intravenous Assay. The results of this study are recorded in Table IV. Each compound evaluated in this assay exhibited salidiuretic activity at 5 mg/kg stat. The most active compound was **4d**, followed closely by **4e** and **4f**. Each compound was more active at this dose than hydrochlorothiazide, except **4a**, but none were as active as furosemide or MK-196 in this assay.

Again, when **4f** is compared with the 3-deoxo analogue (compound **12b** from the earlier study),[1] **4f** seems to be the less active of the two.

(b) Oral Assay. The results of this study are recorded in Table V. Of the three compounds evaluated by this method, only **4b** and **4e** were active. However, they were considerably less acctive than hydrochlorothiazide, furosemide, and MK-196.

Chimpanzee Assays. The results of this study are recorded in Table VI.

Salidiuresis. Every compound in the series was evaluated in this assay. Each indan-1,3-dione was initially tested orally, followed immediately by an intravenous assay. Thus, the effects of the latter study were superimposed on the former. It should be pointed out that the judgments have to be made on the basis of two sequential experiments in one animal, but data obtained in this difficultly accessible species are generally quite reliable.

All but one of the seven 5-oxyacetic acids (**4a–g**) were active. Among the 6,7-dimethyl compounds, **4b** appeared to be somewhat more active than **4a**, suggesting a beneficial contribution made by the second 2-ethyl group. Among the three 6-methyl-7-chloro compounds (**4c–e**), the relative order of activity appears to be **4c** > **4e** > **4d**, suggesting the following activity-enhancing effects of the 2-position substituents: 2-methyl-2-cyclopentyl > 2,2-diethyl > 2,2-spirocyclopentyl. Compound **4g**, the 3-oxo derivative of MK-196, possesses only nominal activity at the doses tested, which are one-fifth and one-half those of the other members of the series. The other 6,7-dichloro compound, **4f**, exhibited activity in the same range as compounds **4b–e**. This is surprising, since in the earlier studies[1,2] the relative activity contributed by the 6- and 7-position substituents was generally as follows: 6,7-dichloro > 6-methyl-7-chloro > 6,7-dimethyl.

Compound **8** an anisole, was inactive. Compound **14**, the tetrazole analogue of **4d**, although inactive orally, displayed about the same response when given intravenously as the corresponding carboxylic acid (**4d**).

The more active indan-1,3-diones exhibited salidiuretic activity comparable to that of hydrochlorothiazide when compared at the same dose. However, they were considerably less efficacious than furosemide or MK-196.

Uricosuric Activity. Considering the uricosuric effects elicited by each compound when given both orally and intravenously, their relative activities fall roughly in the following order: **4c** > **4a** > **4e** > **4b** > **4d** ≫ **4f**. As observed in other series, the salidiuretic and the uricosuric activity did not run parallel. Thus, the uricosuric/salidiuretic ratio varied considerably. Compound **8** was virtually inactive. Compound **4g**, at the very low dose tested, possessed only weak activity. Compound **14** was equally uricosuric with its carboxylic acid counterpart **4d**.

None of the compounds were as uricosuric at the doses tested as MK-196. As expected, hydrochlorothiazide and furosemide were either inactive or retained uric acid. Under the test conditions, **4c** was less uricosuric than its 3-deoxy counterpart (compound **12f** from the earlier paper).[1] The 3-deoxy analogue of MK-196, **4g**, although tested at a lower dose, possessed much weaker uricosuric activity.

In summary, the introduction of a 3-oxo group into (5-indanyloxy)acetic acids that possess salidiuretic activity generally produced compounds that were equipotent with the 1-oxo compounds in rats but with reduced salidiuretic activity in dogs and chimpanzees. However, direct comparisons of compounds with identical substituents in each position, except for the 3-position, was possible only in a few instances.

The uricosuric activity of the compounds in this 1,3-

Table III. Oral Rat Diuretic Activity[a]

	mequiv of electrolyte × 100/cage for the following doses										urine vol, mL, at the following doses			
	Na^+			K^+			Cl^-							
compd	3 mg/kg	9 mg/kg	27 mg/kg	81 mg/kg	3 mg/kg	9 mg/kg	27 mg/kg	81 mg/kg	3 mg/kg	9 mg/kg	27 mg/kg	81 mg/kg	3 mg/kg	9 mg/kg	27 mg/kg	81 mg/kg
4c	19	41	50	76	21	24	22	36	17	30	40	86	24	27	27	25
4d	14	26	35	47	23	30	32	29	10	15	30	61	29	29	33	29
4e	70	92	158	251	29	43	62	106	82	124	222	369	27	26	33	38
4f	50	80	101	164	46	35	43	89	64	97	130	250	30	29	27	31
furosemide	57	69	150	253	31	39	60	97	71	96	228	328	26	28	36	46
HCT[b]	127	133	168	154	50	33	61	61	146	143	191	163	37	30	32	33
MK-196	86	97	129	189	32	50	61	85	85	163	223	303	28	29	31	35
placebo	(27)				(21)				(23)				(26)			

[a] Female rats (Charles River, 150–170 g) were maintained overnight on a sugar diet with water ad libitum. The test substance was dissolved in pure DMF and subsequently diluted with water (which contained 3 drops of Tween 80/100 mL) such that the final vehicle was 4% DMF. At the time of the test, animals were given the vehicle (as placebo) or test substance suspended in a final volume of 5.0 mL po. Rats were housed in groups of three in metabolism cages. Urine was collected for the 0- to 5-h interval in graduated cylinders and was analyzed for sodium, potassium, and chloride content. Animals that received placebo were run concurrently. Results are reported as milliequivalents times 100 per cage and are the geometric means of three cages per dose level. Standard methodology was used for determination of electrolyte levels. [b] HCT = hydrochlorothiazide.

Table IV. Intravenous Dog Diuretic Assay (5 mg/kg Stat)[a]

		control/drug treatment results			
		μequiv/min			urine vol,
compd	no. of animals	Na^+	K^+	Cl^-	mL/min
4a	2	22/106	10/21	4/42	1/2
4b	2	37/196	5/24	6/114	1/4
4c	2	35/132	10/41	5/48	2/2
4d	2	24/305	5/23	9/245	2/5
4e	2	79/259	13/27	6/157	1/2
4f	2	42/247	5/7	8/179	1/2
14	4	59/193	13/27	5/11	2/3
HCT	3	8/166	15/33	5/156	1/3
furosemide[b]	2	29/960	18/141	1/1081	1/3
MK-196	3	107/920	11/62	62/1010	2/8

[a] Conditioned female mongrel dogs, weighing approximately 20 kg in the postabsorptive state, were starved overnight and given 500 mL of water orally 1 h prior to induction of anesthesia with sodium pentobarbital (30 mg/kg iv). After anesthesia was induced, each dog was catherized and primed with creatinine (4 g as a 10% aqueous solution) injected sc in multiple sites. Prior to the initiation of clearance studies, 1.5 mL/kg of isoosmotic pH 7.4 phosphate buffer solution (20 mg of PO_4/kg) was given iv as a priming injection; 3 mL/min of isoosmotic pH 7.4 buffer containing 4% mannitol (6.9 mg of PO_4/min) was infused during the experiment. At the onset of timed clearances, the urinary bladder was emptied and replicate 15-min urine collections were made; venous blood samples were drawn at the midpoint of each period. Following this control phase, the test compound was given iv at 5 mg/kg over a 5-min period, and 15-min collections of urine were taken over a period of 2 h. Urinary electrolytes were assayed by standard methods. The data recorded were the average of the two highest consecutive 15-min collections. [b] 1 mg/kg.

Table V. Oral Dog[a] Diuretic Assay (5 mg/kg)

		mequiv			
compd	no. of animals	Na^+	K^+	Cl^-	urine vol, mL
4b	3	6	5	8	553
4d	3	2	1	3	253
4e	3	7	5	10	570
HCT	50	18	7	25	556
MK-196	12	12	2.2	26	267
furosemide	23	31	8	36	700
placebo	50	1.6	1.1	1.1	225

[a] Oral tests were carried out on a colony of trained female mongrel dogs weighing 8–10 kg. All dogs received 100 mL of water the previous day and were fasted overnight. On the day of the test, 250 mL of water was administered orally, followed by 500 mL of water (orally) 1 h later. One hour after the last oral priming dose of water, bladders were emptied by catheterization, and the study was commenced by administration of compound or placebo. Compounds were given in gelatin capsules, and the animals were maintained in metabolism cages for collection of spontaneously voided urine. Spontaneously voided urine was combined with bladder urine collected by catheterization at the end of 5 h. Urine volumes were measured, and aliquots were analyzed for sodium, potassium, and chloride content by standard methodology. Values are reported as geometric means.

dioxo series was generally about as good as that observed for the same or similar analogues in the 1-oxo series, the exception being the 3-oxo analogue of MK-196 (**4g**) (although **4g** was tested at a lower dose than the other compounds). Therefore, the uricosuric/salidiuretic ratio of these 1,3-dioxo compounds was generally higher than observed with the 1-oxo analogues.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results

Table VI. Oral and Intravenous Chimpanzee Data[a]

compd	administration route	dose, mg/kg	$\Delta C_{urate}/\Delta C_{inulin}$	increase in the no. of μequiv of electrolyte/min Na^+	K^+	Cl^-	increase in vol, mL/min
4a	po	5	0.045	103	32	120	0.6
	iv	1.25	0.258	243	46	288	2.2
4b	po	5	0.061	194	30	244	1.2
	iv	1	0.101	254	41	314	1.6
4c	po	5	0.149	388	54	527	0.0
	iv	1	0.204	82	51	129	0.6
4d	po	5	0.053	170	39	211	−0.5
	iv	1	0.088	59	37	117	1.1
4e	po	5	0.138	290	132	285	2.7
	iv	1	0.082	25	131	123	0.4
4f	po	5	0.007	217	30	226	1.4
	iv	1.25	0.043	187	41	267	2.0
4g	po	1	0.043	2	25	30	0.6
	iv	0.5	0.010	29	31	36	0.3
8	po	5	0.008	4	27	10	0.1
	iv	1	0.000	3	26	13	0.1
14	po	5	0.026	5	2	36	0.0
	iv	1	0.128	119	55	172	0.6
MK-196	po	5	0.332	409	53	554	3.6
	iv[b]	1	0.550	2383	171	2624	14.8
furosemide	po	1	−0.083	738	100	961	7.3
	iv[b]	1	−0.005	749	154	928	0.8
hydrochlorothiazide	po	5	−0.015	144	93	198	1.0
	iv	1.5	0.010	275	25	187	1.8

[a] Fasted male chimpanzees weighing 21–77 kg were immobilized with phencyclidine (which was shown not to affect the results) (1.0–1.5 mg/kg in plus 0.25 mg/kg iv as needed) and were prepared by catheterization for standard renal clearance studies using routine clinical aspectic procedures. Pyrogen-free inulin (iv) was used to measure the glomerular filtration rate (GFR). Clearance of inulin, urate, and the excretion rates of Na^+, K^+, and Cl^- was determined by standard Auto Analyzer techniques. (Inulin and urate in chimpanzee plasma are freely filterable.) Average control clearances were calculated from three 20-min consecutive periods. Drug–response values were derived as the average of eight 15–20-min clearance periods after oral administration of an aqueous solution of the compound through an indwelling nasal catheter. All data are reported as the difference between treatment and control values obtained from single experiments; however, the po data for MK-196 is derived from an average of four experiments. Each compound that was tested both iv and po was evaluated first po and immediately thereafter evaluated iv, unless otherwise specified. [b] This was a separate assay, independent of the po assay.

obtained are within 0.4% of the theoretical values. Detailed experimental procedures are given only for selected compounds, which will serve to illustrate the general synthetic methods employed.

2,2-Diethyl-6-methoxy-4,5-dimethyl-1*H*-indene-1,3(2*H*)-dione (1a). A stirred mixture of 2,3-dimethylanisole (6.8 g, 0.05 mol) and 2,2-diethylmalonyl chloride (10 g, 0.051 mol) in hexane (75 mL) was cooled to 0 °C and treated with $AlCl_3$ (13.5 g, 0.11 mol) in several portions over a 15-min period. The reaction mixture was heated at reflux for 2 h, and then the hexane was removed by distillation at reduced pressure. The residue was treated with cold 1 N HCl, extracted into Et_2O, washed with H_2O and then 5% NaOH, and dried over $MgSO_4$. Evaporation of the Et_2O provided 6.3 g (48%) of **1a**.

2,2-Diethyl-6-hydroxy-4,5-dimethyl-1*H*-indene-1,3(2*H*)-dione (2a). A mixture of **1a** (5.5 g, 0.021 mol) and pyridine hydrochloride (50 g) was heated at 180 °C for 6 h and then poured into ice–H_2O (1 L), providing 4 g (77%) of **2a**.

[(2,2-Diethyl-2,3-dihydro-6,7-dimethyl-1,3-dioxo-1*H*-inden-5-yl)oxy]acetic Acid (4a). Compound **2a** (3.5 g, 0.014 mol), K_2CO_3 (2.2 g, 0.016 mol), and $BrCH_2CO_2Et$ (2.7 g, 0.016 mol) in DMF (20 mL) were heated at 60 °C for 2 h, treated with CH_3OH (40 mL) and KOH (1.1 g, 0.02 mol), heated at reflux for $^1/_2$ h, and then poured into dilute HCl (500 mL), affording 4.2 g (99%) of **4a**.

Methyl [(7′-Chloro-1′,3′-dihydro-6′-methyl-1′,3′-dioxospiro[cyclopentane-1,2′-[2*H*]inden]-5′-yl)oxy]acetate (3). A stirred solution of methyl (2-methyl-3-chlorophenoxy)acetate (3.7 g, 0.0172 mol) and cyclopentane-1,1-dicarbonyl chloride (3.5 g, 0.018 mol) in CH_2Cl_2 (200 mL) was cooled to 0 °C and treated with $AlCl_3$ (7.2 g, 0.054 mol) in several portions during a 15-min period. The reaction mixture was stirred at 25 °C for 18 h and then at reflux for 5 h, cooled, and poured into 1 N HCl. Evaporation of the CH_2Cl_2 at reduced pressure gave 3.6 g (65%) of methyl [(7′-chloro-1′,3′-dihydro-6′-methyl-1′,3′-dioxospiro[cyclopentane-1,2′-[2*H*]inden]-5′-yl)oxy]acetate, which melted at 152–153.5 °C after recrystallization from EtOH–H_2O. Anal. ($C_{17}H_{17}ClO_5$) C, H.

[(7′-Chloro-1′,3′-dihydro-6′-methyl-1′,3′-dioxospiro[cyclopentane-1,2′-[2*H*]inden]-5′-yl)oxy]acetic Acid (4d). Compound **3** (3.5 g, 0.0104 mol), KOH (0.67 g, 0.012 mol), and CH_3OH (200 mL) were combined and heated at reflux for 0.5 h, diluted with H_2O (800 mL), and acidified with HCl to give **4d**.

3,4-Dichloro-5-methoxyphthalic Acid (6). Compound **5** (4 g, 0.015 mol) was suspended in H_2O (200 mL) containing 20% NaOH (1 mL). The mixture was heated to boiling, and $KMnO_4$ (18 g) was added in portions over a 4-h period in such a manner that each time the purple color disappeared an additional portion was added. The MnO_2 was filtered, and the colorless filtrate was acidified with 6 N HCl and evaporated to dryness. The solid residue was extracted with hot Me_2CO, which was evaporated to dryness to give **6**, which melted at 210 °C and was used in step B without further purification.

Dimethyl 3,4-Dichloro-5-methoxyphthalate (7). CH_3I (20 g, 0.2 mol) was added dropwise during a 2-h period to a stirred mixture of **6** (17.5 g, 0.07 mol) and K_2CO_3 (21 g, 0.15 mol) in DMF (175 mL) at 55–60 °C. The reaction was heated for 4 h at 55–60 °C and then poured into H_2O (800 mL) to give 14.5 g (71%) of **7**, which melted at 109–111 °C after recrystallization from Et_2O–C_6H_{14}. Anal. ($C_{11}H_{10}Cl_2O_5$) C, H.

4,5-Dichloro-2-ethyl-6-methoxy-1*H*-indene-1,3(2*H*)-dione (8). To a dispersion of NaH (50% in mineral oil, 0.32 g, 6.6 mmol) in $CH_3C_6H_5$ (20 mL) was added **7** (1.9 g, 6.5 mmol) and 4-heptanone (0.75 g, 6.6 mmol). The reaction mixture was heated at reflux for 18 h and cooled, and the Na salt was filtered, rinsed with C_6H_6, dissolved in H_2O, and acidified with HCl to give 0.9 g (47%) of **8**.

4,5-Dichloro-2-ethyl-6-methoxy-2-methyl-1*H*-indene-1,3-(2*H*)-dione (9). To a stirred solution of **8** (1.3 g, 4.8 mmol) in DMF (15 mL) was added K_2CO_3 (1.26 g, 9.1 mmol) and CH_3I (1.29

g, 9.1 mmol). After 15 min the reaction was poured into H_2O, affording 1.3 g (94%) of **9**.

4,5-Dichloro-6-methoxyphthalic Anhydride (10). A mixture of Ac_2O (150 mL) and compound **6** (23.85 g, 0.09 mol) was stirred and slowly distilled at 125–133 °C at atmospheric pressure over a 3.5- period. After the solution was cooled, 18.75 g (84%) of **10**, which melted at 220–222 °C, was obtained. Anal. ($C_9H_4Cl_2O_4$) C, H.

4,5-Dichloro-6-methoxy-2-phenyl-1*H*-indene-1,3(2*H*)-dione (11). A mixture of **10** (4.94 g, 0.02 mol), $C_6H_5CH_2CO_2H$ (2.72 g, 0.02 mol), Ac_2O (7.55 mL, 0.08 mol), and Et_3N (8.35 mL, 0.06 mol) was heated at reflux for 0.75 h. The volatile constituents were removed at reduced pressure. The residue was treated with 95% EtOH (20 mL), and the solution was heated to reflux, poured into 2% NaOH (450 mL), stirred for 6 h, and filtered. The filtrate was acidified with 6 N HCl to precipitate 4.85 g (76%) of **11**, which melted at 175–178 °C after recrystallization from EtOH. Anal. ($C_{16}H_{10}Cl_2O_3$) C, H.

4,5-Dichloro-6-methoxy-2-methyl-2-phenyl-1*H*-indene-1,3(2*H*)-dione (12). A mixture of **11** (0.5 g, 0.156 mmol), K_2CO_3 (0.432 g, 3.12 mmol), and CH_3I (0.8 mL, 12.5 mmol) in DMF (20 mL) was stirred at 35 °C for 3 h and then poured into H_2O (100 mL) to give 485 mg (93%) of **12**, which melted at 174–175.5 °C after recrystallization from C_6H_{12}–C_6H_6. Anal. ($C_{17}H_{12}Cl_2O_3$) C, H.

[(7′-Chloro-1′,3′-dihydro-6′-methyl-1′,3′-dioxospiro[cyclopentane-1,2′-[2*H*]inden]-5′-yl)oxy]acetonitrile (13). A stirred mixture of **2c** (9.3 g, 0.035 mol), $ClCH_2CN$ (2.83 g, 0.0375 mol), K_2CO_3 (5.2 g, 0.0375 mol), and KI (6.2 g, 0.0375 mol) in DMF (100 mL) was heated at 65 °C for 2.5 h and then poured into H_2O (1.5 L), affording 10.7 g (100%) of **13**, which melted at 137–139 °C after recrystallization from EtOH–H_2O. Anal. ($C_{16}H_{14}ClNO_3$) C, H, N.

4′-Chloro-5′-methyl-6′-(1*H*-tetrazol-5-ylmethoxy)spiro[cyclopentane-1,2′-[2*H*]indene]-1′,3′-dione (14). A stirred mixture of **13** (9.0 g, 0.03 mol), NaN_3 (3.9 g, 0.06 mol), and NH_4Cl (3.2 g, 0.06 mol) in DMF (150 mL) was heated at 95 °C for 2 h and then poured into dilute HCl to give 9.9 g (95%) of **14**, which melted at 211–212 °C after recrystallization from AcOH–H_2O. Anal. ($C_{16}H_{15}ClN_4O_3$) C, H, N.

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Synthesis of Potent Heptapeptide Analogues of Cholecystokinin

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Nine new analogues of acetyl-CCK-heptapeptide (Ac-Tyr(SO_3H)^2-Met^3-Gly^4-Trp^5-Met^6-Asp^7-Phe^8-NH_2) were synthesized by solid-phase methodology. In a first series, the Asp^7 residue was replaced by hydroxy amino acid sulfate esters. In another series, Gly^4 was substituted by D-Ala, while Trp^5 and Met^6 were replaced by their D enantiomer. The introduction of the sulfate ester was performed with a new, mild, crystalline, and stable reagent, pyridinium acetyl sulfate. Each analogue that contained Tyr(SO_3H)^2 and a hydroxy amino acid sulfate ester [Ser(SO_3H), Thr(SO_3H), or Hyp(SO_3H)] in position 7 proved to be more potent (1.9, 1.7, and 3.0 times, respectively) than CCK-8 in vitro (isolated gallbladder strips). While devoid of gastrin-like activity in vivo, these analogues had potent anticonvulsive activity. The analogues containing a D-amino acid residue were less potent than the parent compound in vitro. The D-Ala^4 replacement, however, yielded a compound that was 40% as potent as CCK-8 in the in vitro test but showed prolonged duration of action on sphincter Oddi. While the 7-substituted Ac-CCK heptapeptides are among the most potent CCK analogues reported so far, the D-Ala^4 replacement resulted, for the first time, in prolonged activity in vivo.

The gastrointestinal peptide hormone cholecystokinin-pancreozymin exists in different molecular forms, including CCK-39, CCK-33, CCK-12, and CCK-8.[1] All biologically active CCK fragments contain a tyrosine *O*-sulfate in position 27. The active center of the molecule is the C-terminal heptapeptide[2] (CCK-27-33 or CCK-7), but the shortest naturally occurring biologically active fragment is the C-terminal octapeptide[3] (CCK-26-33 or CCK-8).[4] The N-terminal amino group of CCK-7, however, is not necessary for cholecystokinetic activity,[5] and similarly, the *N*-acetyl derivative of CCK-7 shows the same potency as CCK-8 in the pancreas amylase release test.[6] On the basis of these observations and an earlier study[7] that demonstrated that the aspartyl residue of the tetragastrin (H-Trp-Met-Asp-Phe-NH_2) could be replaced by the electronically equivalent serine *O*-sulfate, we replaced the Asp^7 residue of Ac-CCK-7 by the sulfate ester of serine, threonine, or 3-hydroxyproline. Since it was also reported that

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